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Evaluation of Serological Screening of Cadaveric Sera for Donor Selection for Cornea Transplantation

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Human corneas are explanted for grafting as late as 72 h after death, for example, from medical examiner cases. Currently, infection of the donor with human immunodeficency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) is excluded in most cornea banks by serological testing of the cadaveric serum only. The reliability of this strategy was investigated by testing paired cadaveric and premortem sera of 33 potential donors. Results were discordant in 17 of 33 donors by at least one assay. Most frequently, HBsAg enzyme-linked immunosorbent assay (ELISA) yielded false-positive results with the cadaveric serum (16 of 33 serum pairs). Virus safety of the graft was affected in a single case, which was HCV antibody negative in the cadaveric serum, but positive in the premortem serum (confirmed by HCV-RIBA strip immunoassay). Forensic DNA profiling by polymerase chain reaction (PCR) of both serum samples confirmed that these were derived from the same individual. In conclusion, the results indicate that serological testing of cadaveric sera is not a reliable method for screening of potential cornea donors, and may not be sufficient for the virus safety of cornea grafts. Therefore, other screening strategies such as detection of viral nucleic acids by PCR should be evaluated. ***J. Med. Virol. 58:291–295, 1999.***

KEY WORDS: HIV; HBV; HCV; serology (postmortem); cornea grafts

INTRODUCTION

Allogenic transplantation of the human cornea is feasible and current practice with corneas explanted from donors (e.g., medical examiner cases) as late as 72 h after death. Cornea grafts have transmitted various bacteria and fungi, neurotropic infectious agents (e.g., rabiesvirus and the Creutzfeld–Jacob disease infectious agent), and systemic virus infections, including hepatitis B virus (HBV) and cytomegalovirus [Houff et al., 1979; Eastlund, 1995; Hoft et al., 1997]. Therefore, contraindications for acceptance of corneal tissue before enucleation were agreed at the third European Corneabank Conference in 1990, and published by the European Eye Bank Association (EEBA) [EEBA, 1997]. Contraindications include seropositivity for HBV and human immunodeficiency virus (HIV), and seropositivity for hepatitis C virus (HCV) was proposed as a future contraindication [EEBA, 1997]. At present, most European eye banks test for HBV, HCV, and HIV, and some eye banks test for other infectious diseases, such as syphilis, which was also used previously as a surrogate marker for the risk of HIV infection [Glasser, 1998]. Because detailed guidelines for conducting serological tests have not been set up yet [EEBA, 1997], serological testing of potential cornea donors similar to blood donors and other organ donors is the preferred strategy of most eye banks [Glasser, 1998]. However, in contrast to blood donors and other organ donors, only cadaveric sera are available in most cases of cornea donors. Because most commercially available serological tests are neither evaluated nor licensed for testing cadaveric sera by national agencies, for example, the U.S. Food and Drug Administration (FDA) or the German Paul-Ehrlich-Institut (PEI), serological screening of potential cornea donors holds major drawbacks. A study was undertaken to determine whether serological testing of cadaveric sera for HIV, HBV, and HCV is sufficient to screen for virus safety of cornea transplants.

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TABLE I. Potential Cornea Donors With Results of Serological Tests in the Cadaveric Serum Concordant With the Last Premortem Serum Sample

Potential donor #	t_1 (days)	t_2 (h)	Age (years)	Sex	Serology (premortem serum/cadaveric serum) HBsAg	HCV	HIV
1	1	19	53	M	pos/pos	neg/neg	neg/neg
2	1	60	65	M	neg/neg	neg/neg	neg/neg
3	2	11	46	M	neg/neg	neg/neg	neg/neg
4	1	47	49	F	neg/neg	neg/neg	neg/neg
5	1	12	60	M	neg/neg	neg/neg	neg/neg
6	12	29	55	M	neg/neg	neg/neg	neg/neg
7	<1	24	73	M	neg/neg	neg/neg	neg/neg
8	9	21	52	M	neg/neg	neg/neg	neg/neg
9	15	22	85	F	neg/neg	neg/neg	neg/neg
10	2	27	47	M	neg/neg	neg/neg	neg/neg
11	1	12	47	F	neg/neg	neg/neg	neg/neg
12	4	13	34	M	neg/neg	neg/neg	neg/neg
13	8	15	83	M	neg/neg	neg/neg	neg/neg
14	1	20	64	M	neg/neg	neg/neg	neg/neg
15	1	27	65	F	pos/pos	neg/neg	neg/neg
16	17	15	63	M	neg/neg	neg/neg	neg/neg

t_1, time elapsed between collecting of the last premortem serum sample and death (days); t_2, time elapsed between death and collection of the cadaveric serum sample (h); HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

MATERIALS AND METHODS

Study Design

Cadaveric serum samples and premortem serum samples of 33 cornea donors were collected between November 1996 and February 1998 and tested comparatively for HIV and HCV antibodies, and hepatitis B surface antigen (HBsAg). The time interval between collecting of the premortem serum and death (t_1) was less than 17 days. Premortem serum samples were obtained in 17 potential donors from blood samples delivered to the department of clinical chemistry by the hospital's emergency unit less than 24 h before death. In 16 potential donors, premortem serum samples were delivered directly to the institute for virology 1–17 days before death, for example, by the emergency unit or during a previous hospitalization of the patient. Cadaveric serum was collected at the time of cornea explantation from the vena jugularis. Time elapsed between death and collection of the cadaveric serum (t_2) was less than 60 h.

Serology

Commercially available serological tests, which were licensed in Germany for the screening of blood donors by the PEI (Langen, Germany), were used according to the manufacturers' instructions: HCV 3.0 ELISA Test System SAVe (Ortho Diagnostic Systems, Neckargemünd, Germany; PEI # 193a/92), Murex HBsAg Enzyme Immunoassay for the Detection of Hepatitis B Surface Antigen (PEI # 109a/94), ICE* HIV-1.0.2 Detection Pack (PEI # 72a/95) with ICE* Base Pack (all three: Murex Diagnostika, Burgwedel, Germany). Positive results were confirmed twice with the same assay system. A strip immunassay, RIBA HCV 3.0 SIA (Chiron, Emeryville, CA) was carried out for confirmation of positive HCV antibody screening results. Confirmatory tests were not conducted with cadaveric serum samples because the FDA does not accept this strategy [Glasser, 1998].

HCV Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

For the detection of HCV RNA, nucleic acids were extracted from the serum using the Amplicor Specimen Preparation Kit. Amplification was performed using the Amplicor HCV Amplification Kit, and the amplification product was detected by hybridization with the Amplicor HCV Detection Kit (all kits: Hoffmann-LaRoche, Grenzach-Wyhlen, Germany).

Genomic DNA Profiling

DNA fom both the cadaveric and the premortem serum sample was genotyped using the AmpFLP Profiler Amplification Kit (PE Applied Biosystems, Weiterstadt, Germany). This multiplex PCR detects simultaneously nine evaluated short tandem repeat loci and the amelogenin marker for sex differentiation by using different fluorescent dyes for labeling fragments in overlapping size ranges [Fregeau and Fourney, 1993; Kimpton et al., 1993]. Fragments were separated and detected by capillary electrophoresis (ABI Prism 310; PE Applied Biosystems).

RESULTS

Concordant results of the HBsAg, HCV, and HIV assays in both the cadaveric and the premortem serum sample were noted in 16 of 33 potential cornea donors (Table I). Thus, two HBsAg positive potential donors (#1 and #15, Table I) were identified correctly by the

TABLE II. Potential Cornea Donors With Results of Serological Tests in the Cadaveric Serum Divergent From the Last Premortem Serum Sample

Potential donor #	t_1 (days)	t_2 (h)	Age (years)	Sex	Serology (premortem serum/cadaveric serum) HBsAg	HCV	HIV
17	<4	12	39	F	neg/pos	neg/neg	neg/neg
18	1	20	75	M	neg/pos	neg/neg	neg/neg
18	<1	25	57	M	neg/pos	neg/neg	neg/neg
20	5	48	69	M	neg/pos	neg/neg	neg/neg
21	1	46	61	F	neg/pos	neg/neg	neg/neg
22	1	14	67	M	neg/pos	pos/neg	neg/neg
23	1	15	73	M	neg/pos	neg/neg	neg/neg
24	1	5	54	F	neg/neg	neg/neg	neg/pos*
25	1	24	76	M	neg/pos	neg/neg	neg/pos
26	1	29	54	F	neg/pos	neg/neg	neg/neg
27	3	29	39	M	neg/pos	neg/neg	neg/neg
28	6	21	51	F	neg/pos	neg/neg	neg/neg
29	13	36	65	F	neg/pos	neg/neg	neg/neg
30	1	21	66	M	neg/pos	neg/neg	neg/neg
31	5	19	67	M	neg/pos	neg/neg	neg/neg
32	5	27	42	M	neg/pos	neg/neg	neg/neg
33	17	17	79	F	neg/pos	neg/neg	neg/neg

t_1, time elapsed between collecting of the last premortem serum sample and death (days); t_2, time elapsed between death and collection of the cadaveric serum sample (hours); HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

*Sample addition verification system indicated inadequate conditions due to the hemolytic colour of the cadaveric serum sample.

strategy of screening cadaveric sera. On the other hand, 17 of 33 serum sample pairs had divergent results in at least one of the serological tests (Table II). The time elapsed between collecting the last premortem serum and death did not differ significantly between the group of cornea donors with discordant serology results and the group of potential cornea donors with concordant results in the cadaveric and premortem serum samples (3.9 days, standard deviation [SD] ± 4.6 *vs.* 4.8 days, SD ± 5.6, $P = .63$; Student's t-test). In addition, there was no significant difference ($P = .88$, Student's t-test) between both groups concerning the time (t_2) elapsed between death and collecting of cadaveric serum (23.4 h, SD ±13.3 h; Table I *vs.* 24 h SD ±11.3, Table II).

In 16 of 17 potential donors with divergent results in the cadaveric serum sample from the premortem serum sample, the results of the HBsAg assay were positive in the cadaveric, but negative in the premortem serum sample. Furthermore, the HIV ELISA was positive in two cadaveric serum samples, whereas the corresponding premortem serum sample was HIV antibody negative. In one of these two cadaveric serum samples (donor #24, Table II), the sample addition verification system of the HIV antibody assay indicated inappropriate conditions, obviously due to the autolysis/hemolysis of the cadaveric serum sample.

In potential donor #22 (Table II), the current strategy of serological screening of cadaveric serum samples may have affected the virus safety of the cornea graft, since the HCV antibody ELISA gave a negative result with the cadaveric serum sample, but a positive result with the paired premortem serum sample. Both results were confirmed by repeating the HCV ELISA twice and by HCV RIBA (Fig. 1). In addition, HCV RT-PCR was carried out with the cadaveric and the premortem serum sample of donor #22. Both serum samples were HCV-RNA negative. Comparative forensic DNA profiling via PCR of both the cadaveric serum and the premortem serum, which was delivered from another laboratory (Department of Clinical Chemistry), obtained an identical genotype for both DNA specimens with a P-value of 3.73×10^{-12} (i.e., the probability that two individuals selected at random will have matching genotypes). Thus, DNA genotyping undoubtedly confirmed that both samples were derived from the same individual.

DISCUSSION

Serological testing of cadaveric serum samples, which are the only available serum samples in most potential cornea donors, is currently the accepted strategy to prevent the transmission of systemic viral infections (e.g., infections with HBV, HCV, HIV) by cornea grafts [Glasser, 1998]. Although the commercially available serological tests are only evaluated and licensed for screening of premortem serum samples of blood donors, these tests are used frequently by eye banks for screening of cadaveric sera, for example, in 400,000 cornea donors in the U.S. during the past 12 years [Glasser, 1998]. The applicability of serological testing of cadaveric sera for HIV has been indicated by a study that screened cadaveric sera of AIDS patients and patients at high risk for HIV infection by ELISA and Western blot [Pepose et al., 1987b]. Thus, a 94% to 99% sensitivity of HIV antibody test kits for cadaveric sera was established compared to the autopsy diagnosis of AIDS. However, a direct comparison of HIV se-

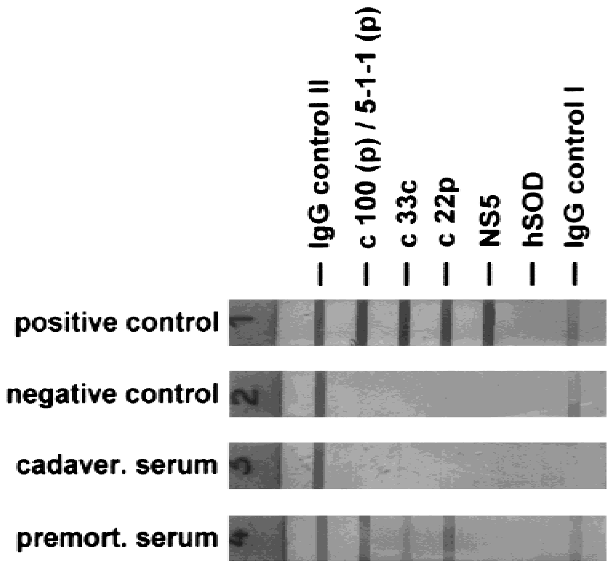

Fig. 1. Chiron Riba HCV 3.0 strip immunoblot assay of the cadaveric and premortem serum of potential donor #22.

rology with paired premortem serum samples had not been carried out so far, obviously because premortem serum samples of potential cornea donors are difficult to obtain. In addition, there is only a single study that evaluated the use of an HCV ELISA for cadaveric sera of potential cornea donors [Laycock et al., 1997].

At least we demonstrated that a cadaveric serum sample of an HCV antibody positive potential cornea donor (#22) gave a negative result, thus affecting the virus safety of the cornea transplant procedure. Interestingly, both ELISA and RIBA failed to detect HCV antibodies in the cadaveric serum sample. As the premortem serum sample of the potential donor #22 was not delivered directly to the virology laboratory, but derived from a blood sample sent from the emergency ward to the Department of Clinical Chemistry, incorrect identification of the sample may be suspected. Hence, forensic DNA profiling by detecting various short tandem repeat loci was conducted, which is a highly reliable method for confirming the identity of various human biologic materials [ISFH, 1994]. The very low probability for the genotypes of both sera matching at random ($P = 3.73 \times 10^{-12}$) demonstrated that both the cadaveric serum and the premortem serum were derived from the same individual. Therefore, it is concluded that the HCV serology of donor #22 was false negative. In contrast to ELISA, RIBA indicated incompletely adequate conditions by a faint IgG control band I (cadaveric serum, Fig. 1). An explanation for the false-negative result with the cadaveric serum sample may be hemodilution due to receiving crystalloids, colloids, or blood products shortly before death [Heck and Baxter, 1994; LeFor et al., 1995]. Another possible explanation for the false-negative HCV serology may be the interference of autolysis/hemolysis products or microbial contaminants of the cadaveric serum with the serological assays. Although both serum samples of donor #22 were negative for HCV-RNA, infectivity (estimated by HCV-RNA positivity) cannot be excluded, since the sensitivity of the Amplicor HCV RT-PCR was never evaluated for cadaveric serum samples. Moreover, the premortem serum sample was not handled and stored as proposed by the RT-PCR kit's instructions because the serum was not delivered directly to the laboratory. Fortunately, potential donor #22 would have been rejected with the current screening strategy by a false-positive result of the HBsAg ELISA.

In contrast to the HCV serology, (false-)positive results were noted with the HBsAg ELISA in 16 cadaveric sera, and with the HIV ELISA in 2 cadaveric sera, whereas the paired premortem sera were HBsAg negative and HIV negative. A true seroconversion of these potential donors is highly improbable, because the average time (t_1) beween collection of the premortem serum and death was very short, 3.9 days (SD ± 4.6), and the incidence of HBV infections and HIV infections (seroconversions) in Germany is very low, 50 and 5.9 per 10^5 persons and year, respectively [Lange and Masihi, 1987; Robert-Koch-Institut, 1998]. Therefore, a false-positive reaction by HBsAg ELISA or HIV ELISA is the most probable explanation for the discordant results compared with premortem sera, and an interference of hemolysis/autolysis products of the cadaveric serum with the assay system can be suspected. However, the accumulation of interfering hemolysis/autolysis products after death seems to be highly variable and not related directly to the time (t_2) elapsed between death and collecting the cadaveric serum, as there was no significant difference in t_2 between the group with false-positive results and the group with concordant results.

Similar to the above results, a low specifity and a low predictive value of HBsAg ELISA testing of cadaveric sera compared to radioimmunoassays and to confirmatory (HBsAg neutralization) tests had been reported recently [Pepose et al., 1992; Migden et al., 1996], but a direct comparison with premortem serum samples was not carried out. Because the FDA does not accept the validity of confirmatory HBsAg tests in cornea donors, the high rate of obviously false-positive HBsAg ELISA results leads to the loss of valuable corneas [Glasser, 1998] but does not affect the virus safety of the cornea graft. By contrast, false-negative HBsAg results with cadaveric serum samples would affect the safety of the current cornea transplant strategy, because HBV was transmitted effectively by cornea transplantation [Hoft et al., 1997].

On the other hand, no data are available on the erroneous transplantation of corneas from HCV positive donors, and several cases of transplation of corneas from HIV-positive donors have been reported that did not result in an infection of the cornea recipient [Pepose et al., 1987a; Schwarz et al., 1987]. This finding indicates that the risk of HIV transmission by a cornea transplant may be rather low, although HIV can be isolated from the cornea and tears [Salahuddin et al., 1986; Ablashi et al., 1987]. At least no case of HCV or

HIV transmission through >400,000 cornea transplants has been reported during the past 12 years [Glasser, 1998]. However, a systematic search for HCV and/or HIV seroconversions in cornea recipients has not yet been carried out to our knowledge. As HCV and HIV infections tend to be asymptomatic for several years, infections of cornea recipients may go undetected without a systematic search.

In conclusion, the results indicate that serological testing of cadaveric sera may not be sufficient for the safety of cornea grafting, and other strategies should also be performed in the future. These strategies may include careful preselection of potential donors, systematic collection of premortem serum of potential donors, and PCR testing of the graft, blood, or other appropriate tissues (e.g., lymph nodes and liver).

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